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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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14. ABSTRACT The work in this study addresses the development of post-traumatic osteoarthritis (PTOA) and seeks to develop a basis for future therapeutic interventions. We proposed that increased local inflammation in the C57BL/6 strain contributes to the progression of articular cartilage degeneration following joint trauma with articular fracture. The objective of this project is to locally inhibit early intra-articular inflammation following joint trauma with articular fracture and thereby promote repair and diminish the severity of arthritic changes in articular cartilage and other joint tissues. ELP-IL-1Ra significantly reduced synovial inflammation at 8 weeks after fracture. ELP-IL-1Ra injection resulted in improved synovitis scores of fractured limbs that were not significantly different than control limbs. However, administration of ELP-sTNFRII or ELP- IL-1Ra+sTNFRII showed detrimental effects on bone morphology, cartilage degeneration, and synovial inflammation. The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue. ELP demonstrated utility for an injectable drug depot for clinical intra-articular applications in the treatment of joint trauma.					
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Award Number: W81XWH-10-1-0890

"Development of Intra-Articular Drug Delivery to Alter Progression of Arthritis Following Joint Injury"

PRINCIPAL INVESTIGATOR: Steven A. Olson

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Final Report for Hypothesis Development Award USAMRMC Proposal Number OR090702, Award Number W81XWH-10-1-0890 entitled, "Development of Intra-Articular Drug Delivery to Alter Progression of Arthritis Following Joint Injury"

PI: Steven A. Olson, MD

This final report outlines the research accomplishments for the duration of this hypothesis development award that started on 15 September 2010 and following the request of a one year no-cost extension was completed on 14 April 2013. The current final report was due 14 April 2013. I apologize for the delay in submission of this report.

Introduction

The work in this study addresses the development of post-traumatic osteoarthritis (PTOA) and seeks to develop a basis for future therapeutic interventions. Currently, the only disease-modifying treatment for articular fractures is surgical intervention. Estimates suggest as many as 12% of all symptomatic osteoarthritis patients are post-traumatic [1]. At the present time, the state of the art treatment for displaced articular fractures in weight bearing joints is surgical stabilization of the fracture with anatomic restoration of the articular surface when possible. Even with optimal treatment, displaced articular fractures in the lower extremity have a 10-20% incidence of post-traumatic osteoarthritis [2]. Currently, there is no pharmacological intervention, either systemically or locally, that has been shown to decrease the incidence of post-traumatic osteoarthritis. Growing evidence suggests that the inflammatory environment of the joint may also play a critical role in the development and progression of post-traumatic arthritis.

Preliminary studies leading to this project introduced a novel model of a closed intra-articular fracture in C57BL/6 mice that develops PTOA[3]. Acute joint pathology and inflammation in this model were found to increase with increasing injury severity[4]. Ward found that the MRL/MpJ strain of mice is protected from development of PTOA[5]. Lewis et al reported on the genetic and cellular evidence that decreased inflammation in the MRL/MpJ is associated with protection from PTOA after acute injury[6]. For this work we follow-up on the observation that increased local inflammation in the C57BL/6 mouse strain contributes to the progression of articular cartilage degeneration following joint trauma with articular fracture. A more thorough understanding of the role of inflammation following articular fracture may provide the basis of novel therapeutics for enhancing regeneration.

We proposed that increased local inflammation in the C57BL/6 strain contributes to the progression of articular cartilage degeneration following joint trauma with articular fracture. The objective of this project is to locally inhibit early intra-articular inflammation following joint trauma with articular fracture and thereby promote repair and diminish the severity of arthritic changes in articular cartilage and other joint tissues.

Methods

We used a murine model of a closed articular fracture in a C57BL/6 strain for this investigation. We are using a thermally-responsive drug carrier, ELP, as a drug 'depot' within the joint space that will slowly disaggregate to locally release an anti-inflammatory drug over time[7]. The effect of local inhibition of the inducible intra-articular inflammatory response with ELP-IL-1 receptor antagonist (IL-1Ra) depots or ELP-soluble TNF receptor (sTNFRII) depots following articular fracture was studied in C57BL/6 mice. Following fracture, ELP-IL-1Ra or ELP-sTNFRII depots were intra-articularly administered separately and in combination. Joint tissues were assessed for arthritic changes using MicroCT, histology, and biomarkers at 4 and 8 weeks post-trauma using outcome measures reported in earlier studies [3-6]. A graphic outlining the study design of this investigation is shown in Figure 1. The results and findings are outlined below.

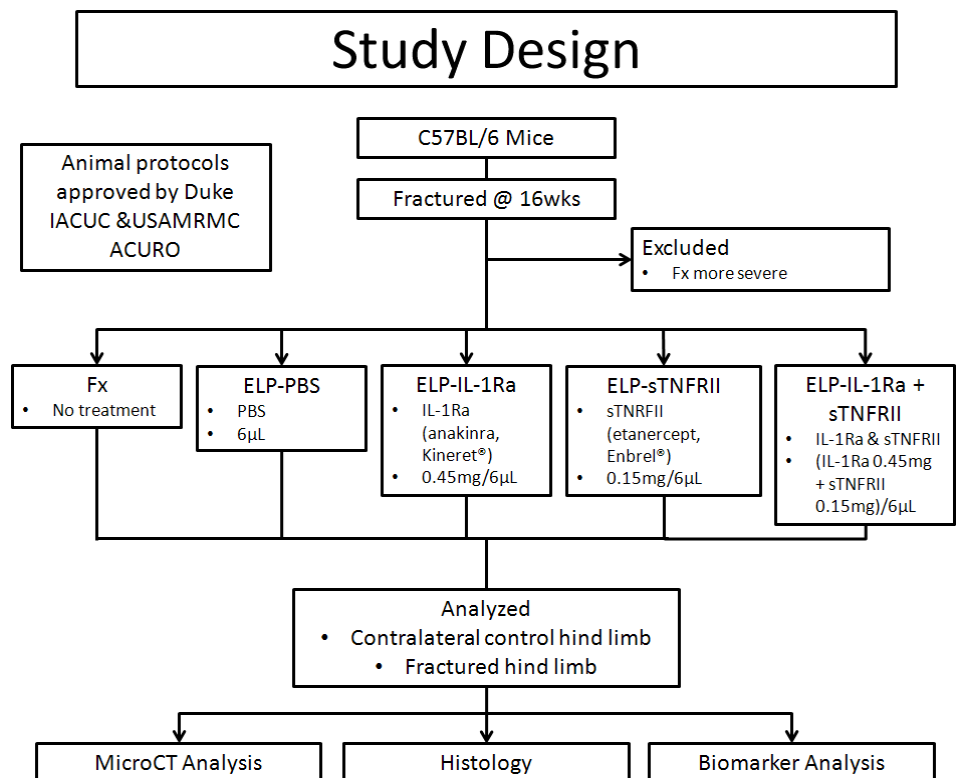


Figure 1: The study design for this investigation is shown in this illustration.

Body

This final report outlines the research accomplishments for the duration of this hypothesis development award. All tasks as outlined in the Statement of Work were completed. To date we have accomplished the successful production of sterile ELP depots. The ELP depots were successfully loaded with the drugs IL-1Ra (anakinra, Kineret®) and sTNFRII (etanercept, Enbrel®). The drug release profiles of IL-1Ra and sTNFRII from the ELP depots were successfully quantified. Closed articular fractures of the tibial plateau were successfully created with an 83% success rate in all mice. Following fracture, intra-articular injections of the ELP depots with IL-1Ra, sTNFRII, IL1Ra+sTNFRII, and PBS were successfully administered in all mice with no complications or adverse events. All animals including age-matched controls with no fracture and mice that received a fracture only with no treatment were sacrificed at 4 and 8 weeks post-fracture. Serum, synovial fluid and both hind limbs were harvested from all animals. Hind limbs from all mice were formalin fixed and scanned by MicroCT. Bone morphological analysis was completed, and all limbs were processed, embedded in paraffin and sectioned for histologic staining. Arthritic changes in the cartilage and synovial inflammation were quantified from histology. Biomarkers were also assessed. Systemic levels of IL-1Ra and sTNFRII were longitudinally quantified in serum from mice.

The major findings of this study were:

- 1) Serum levels of IL-1Ra and sTNFRII were measurable at 5 days following intra-articular injection (Figure 2), which confirmed that ELP functioned as a slow-release drug depot.
- 2) Administration of ELP-sTNFRII or ELP- IL-1Ra+sTNFRII showed a detrimental effect on bone morphology (Figure 3), cartilage degeneration (Figure 4), and synovial inflammation (Figure 5).
- 3) The inhibition of TNF- α resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue.
- 4) ELP-IL-1Ra group reduced cartilage degeneration at 4 and 8 weeks and showed no detrimental effects on bone morphology. ELP-IL-1Ra was the only group in which the Mankin score of the fractured limb was not significantly different from the control limb (Figure 4).
- 5) ELP-IL-1Ra significantly reduced synovial inflammation at 8 weeks. ELP-IL-1Ra was the only group in which synovitis scores of fractured limbs were not significantly different than control limbs (Figure 5). The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue.
- 6) ELP demonstrated utility for an injectable drug depot for clinical intra-articular applications in the treatment of joint trauma.

This study shows a novel reduction in post-trauma inflammation and demonstrates utility for an injectable drug depot for clinical intra-articular applications in the treatment of joint trauma.

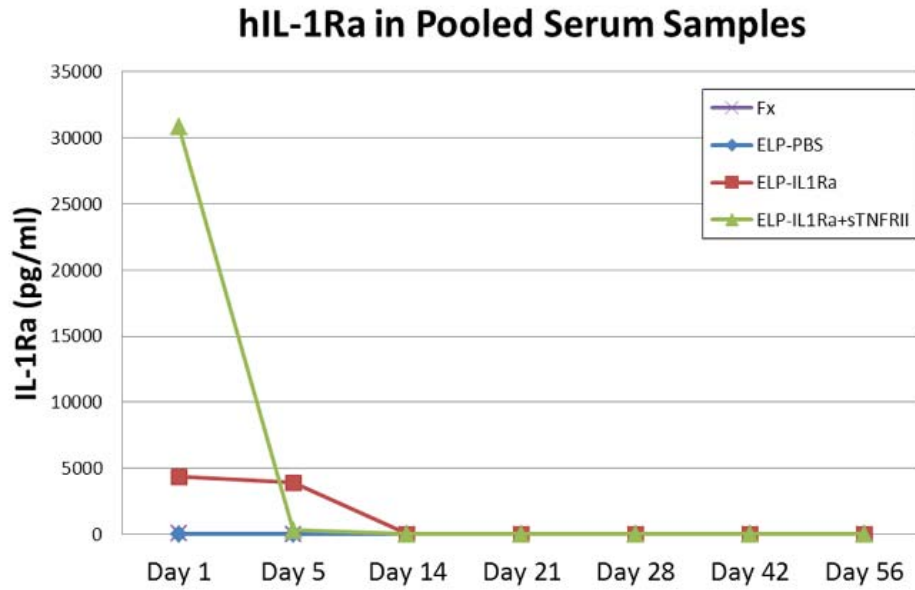
DOD Final Report on Animal Use:

- Species used: mice
- Number of each species used: 112
- USDA Pain Category for all animals used:
- Category C (Non-Painful Procedures): 16
- Category D (Procedures using anesthesia/analgesia): 96

Supporting Data

Figure 2. Drug release rates from longitudinally-collected serum samples. Serum levels of (A) IL-1Ra and (B) sTNFRII were quantified using commercially available ELISA kits (R&D Systems). IL-1Ra and sTNFRII were detectable at least until day 5 post-fracture.

A



B

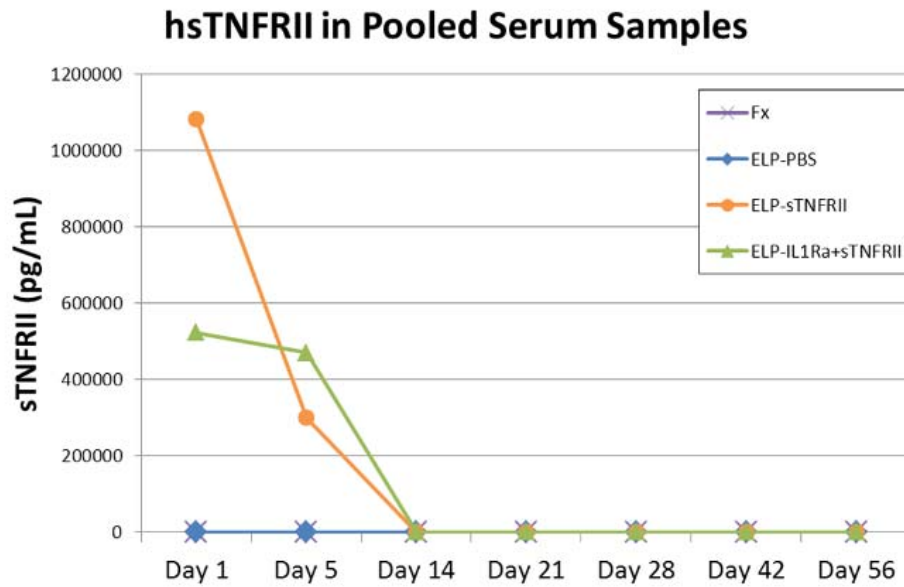


Figure 3. Tibial plateau bone morphology data using MicroCT analysis. Bone volume data shown at (A) 4 weeks and (B) 8 weeks. Bone density data shown at (C) 4 weeks and (D) 8 weeks. (E) Representative MicroCT images of tibial plateau shown for each group at 8 weeks. Scale bar indicates 1mm.

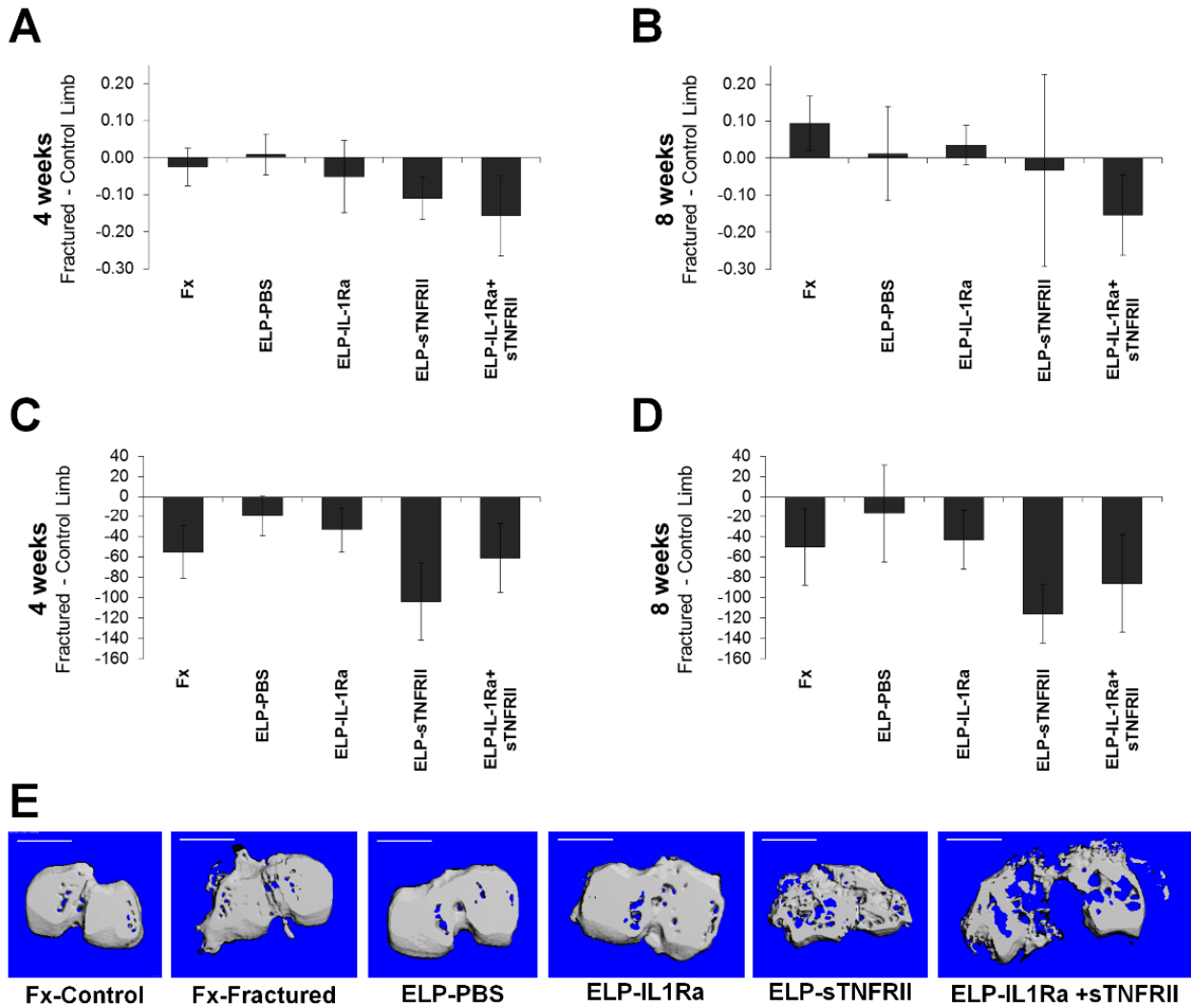


Figure 4. Cartilage degeneration assessment using modified Mankin total joint score and representative MicroCT and Safranin-O/Fast Green stained sections. Modified Mankin total joint score shown for (A) 4 weeks and (B) 8 weeks. P values denote significance between control and fractured limb using Wilcoxon Matched pairs test. Median values and 25th-75th quartile range displayed (n=6-8 per group). (C) Representative histological pictures with MicroCT (blue) and Safranin-O/Fast Green shown for each group at 8 weeks. Scale bars indicate 1mm and 5μm respectively.

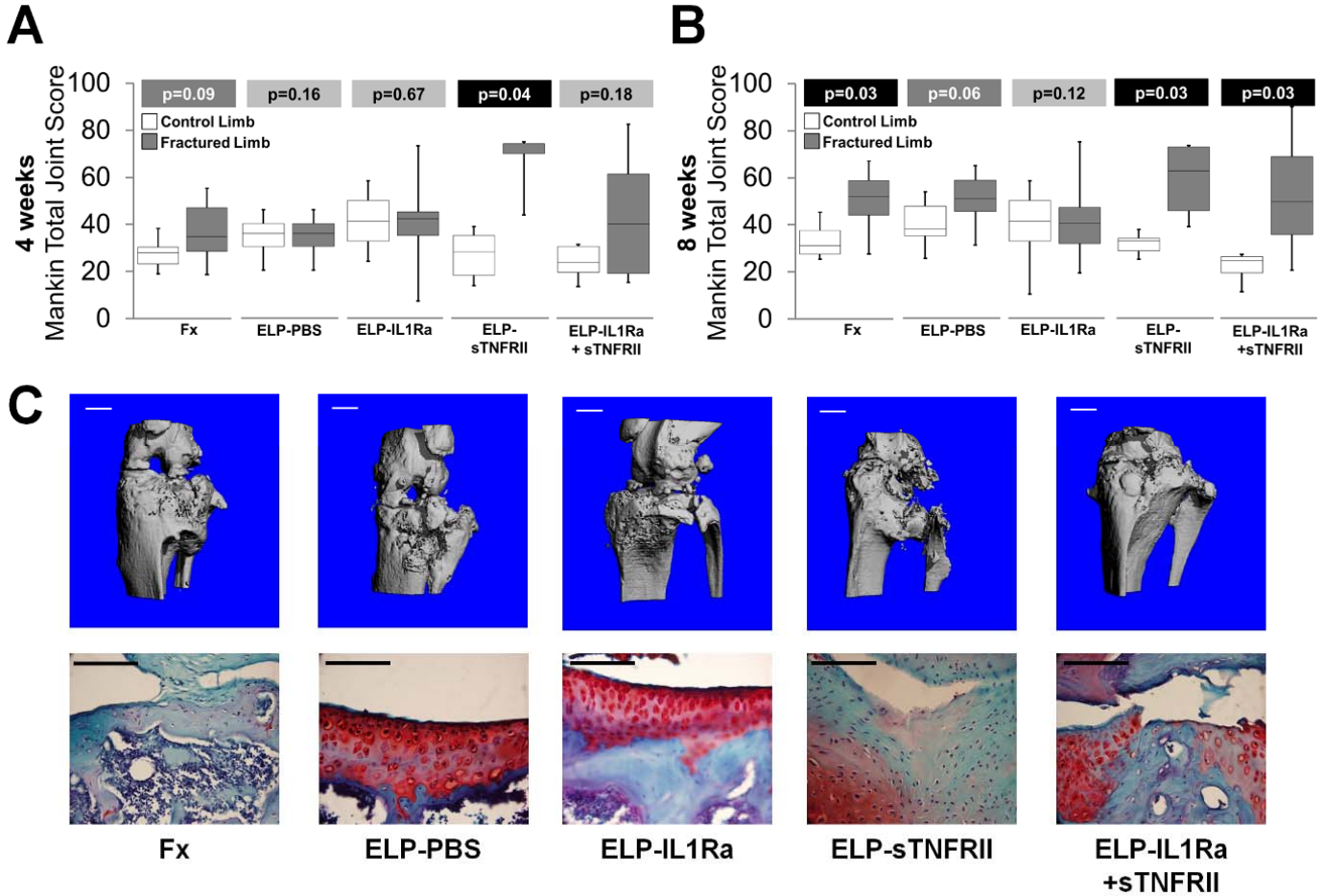
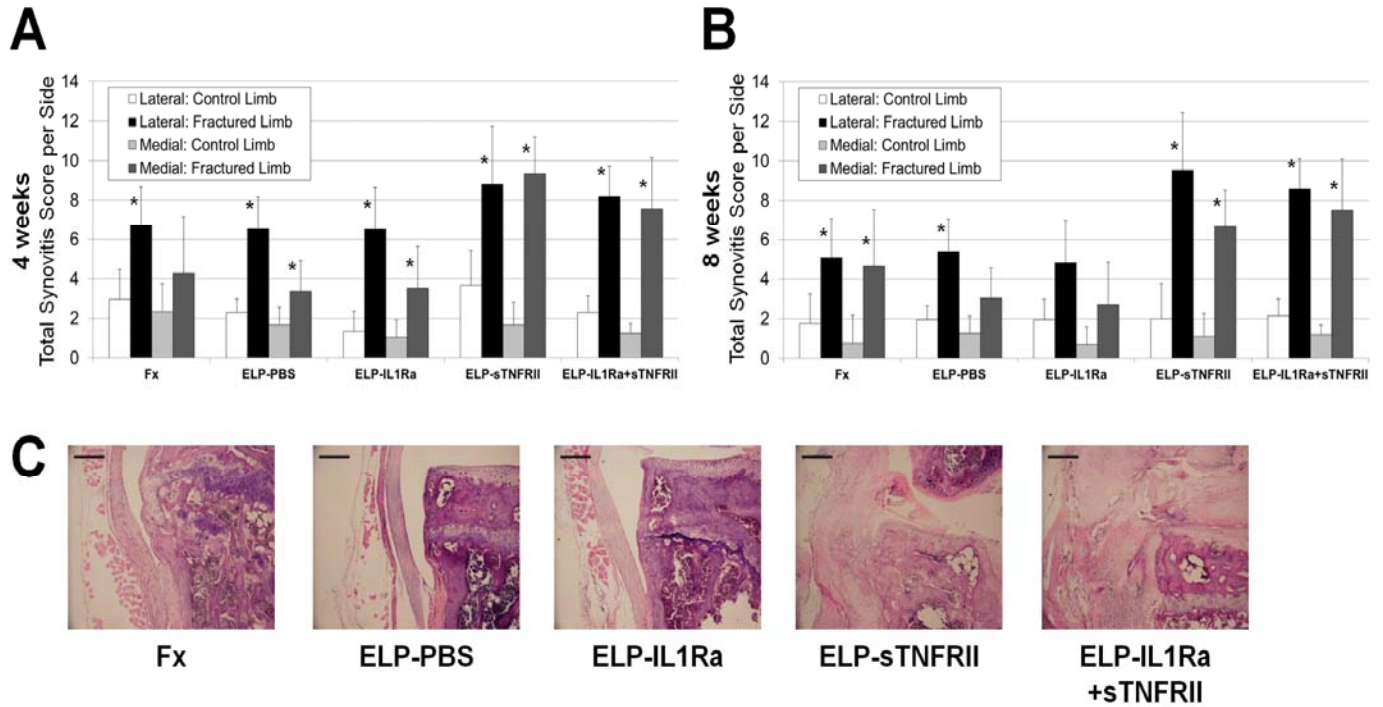


Figure 5. Synovial inflammation assessment using modified Krenn score and representative Hematoxylin & Eosin stained sections. Modified Krenn joint score per lateral and medial side shown for (A) 4 weeks and (B) 8 weeks. * denotes $p < 0.05$ between control and fractured limb using Wilcoxon Matched Pairs test. Mean and standard deviation shown for data ($n = 5-8$ per group). (C) Representative histological pictures of Hematoxylin & Eosin staining shown for each group at 8 weeks. Scale bar indicates $10\mu\text{m}$.



Key Research Accomplishments

- Sterile ELP depots were successfully produced.
- The ELP depots were successfully loaded with the drugs IL-1Ra (anakinra, Kineret®) and sTNFRII (etanercept, Enbrel®).
- The drug release profiles of IL-1Ra and sTNFRII from the ELP depots were successfully quantified.
- Closed articular fractures of the tibial plateau were successfully created in 80 of 96 mice with an 83% success rate.
- Following fracture, intra-articular injections of the ELP depots with IL-1Ra, sTNFRII, IL-1Ra+sTNFRII, and PBS were successfully administered in all mice with no complications or adverse events.
- Animals were sacrificed at 4 and 8 weeks post-fracture. Serum, synovial fluid and hind limbs were harvested from all animals. Hind limbs from all mice were formalin-fixed, scanned by MicroCT, processed, paraffin embedded, and sectioned.
- Bone morphologic, histologic and biomarker analyses were completed.
- Serum levels of IL-1Ra and sTNFRII in mice demonstrated that the ELP successfully functioned as a slow release drug depot.
- Administration of sTNFRII alone or in combination with IL-1Ra resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue.
- The prolonged intra-articular delivery of IL-1Ra reduced the severity of arthritic changes in both cartilage and joint tissue.

Reportable Outcomes

- An abstract entitled “Prolonged Local Delivery of IL-1Ra Prevents Post-traumatic Arthritis in Mice” was accepted at the 2013 World Congress on Osteoarthritis in Philadelphia, PA April 18 – April 21, 2013 and received a podium presentation.
- A manuscript is in preparation for submission to the Journal of Clinical Investigation.
- Based on the promising results supported from this award, a pre-application is being prepared for submission. The purpose of this proposal would be to conduct the preliminary basic and clinical work necessary to translate the use of IL-1Ra into a human clinical trial of patients with lower extremity articular fractures requiring operative treatment.
- Abstracts, presentations, manuscripts: **Abstract submitted and accepted at OARSI 2013, Submitted to Orthopaedic Trauma Association**
- licenses applied for and/or issued; **None**
- degrees obtained that are supported by this award; **None**
- development of cell lines, tissue or serum repositories; **None**
- informatics such as databases and animal models, etc.; **None**
- funding applied for based on work supported by this award; **Submitted preproposal for TRP mechanism for Orthopaedic Peer Review Award; Preparing preproposal for submission to Medical Peer Review Award.**

Conclusion

To summarize, all aspects of the proposed project were successfully completed. Our objectives were to locally inhibit early intra-articular inflammation following articular fracture in the mouse knee and assess the severity of arthritic changes in cartilage and joint tissues long-term.

We were able to show that ELP demonstrated utility for an injectable drug depot for clinical intra-articular applications in the treatment of joint trauma.

Prolonged intra-articular administration of sTNFRII resulted in detrimental bone morphologic, cartilage and synovium changes.

Prolonged intra-articular administration of IL-1Ra following intra-articular fracture reduced the severity of arthritic changes in both cartilage and joint tissue. This finding suggests that local administration of IL-1Ra following joint injury may represent a novel adjunct therapy to surgical stabilization for the prevention of post-traumatic arthritis.

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6. Lewis, J.S., Jr., et al., *Genetic and cellular evidence of decreased inflammation associated with reduced incidence of posttraumatic arthritis in MRL/MpJ mice*. Arthritis Rheum, 2013. 65(3): p. 660-70.
7. Adams, S.B., Jr., et al., *Sustained release of antibiotics from injectable and thermally responsive polypeptide depots*. J Biomed Mater Res B Appl Biomater, 2009. 90(1): p. 67-74.

Appendix I The abstract accepted for podium presentation at the 2013 World Congress on Osteoarthritis. Paper presented in Philadelphia, PA, May 2013

Prolonged Local Delivery of IL-1Ra Prevents Post-traumatic Arthritis in Mice

Kimmerling, KA^{1,2}; Furman, BD¹; Mangiapani, DS¹; Moverman, MA²; Sinclair, SM²; Huebner, JL¹; Kraus, VB¹; Setton, LA^{1,2}; Guilak, F^{1,2}; Olson, SA¹. ¹Duke University Medical Center, Durham, NC, ²Duke University, Durham, NC

PURPOSE: Post-traumatic arthritis (PTA) is defined as osteoarthritis (OA) resulting from joint trauma. Pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF- α) are reported to be upregulated following joint trauma and have been implicated in the pathogenesis of PTA. Presently, surgical restoration is the only treatment for articular fractures. We hypothesize that local sustained inhibition of IL-1, TNF- α , or IL-1 and TNF- α together prevents the development of post-traumatic arthritis following articular fracture. Our objectives were to locally inhibit early intra-articular inflammation following articular fracture in the mouse knee and assess the severity of OA changes in cartilage and joint tissues long-term.

METHODS: All animal procedures were performed in accordance with an IACUC-approved protocol. Male C57BL/6 mice (n=77) were subjected to an articular fracture at 16 weeks of age using an established model. Five groups were established (n=12-16 per group). One group received no treatment after fracture. The remaining groups received intra-articular injections of PBS, IL-1 Receptor antagonist (IL-1Ra; anakinra; Kineret®), soluble TNF receptor II (sTNFRII; etanercept; Enbrel®) or both IL-1Ra and sTNFRII in combination. The treatments were encapsulated in elastin-like polypeptide (ELP) drug depots that have been associated with a 25-fold increase in half-life for intra-articular delivery.

Mice (n=6-8 per group) were sacrificed at 4 and 8 weeks. The left (fractured) and right (non-fractured) limb were harvested and fixed. Micro-computed tomography (MicroCT) of both limbs was performed to assess bone morphology. Joints were processed for standard histology, and sections were assessed by 3 independent blinded graders for cartilage degeneration using a modified Mankin score and synovial inflammation using a modified synovitis score with semi-quantitative scales. Non-parametric statistical analyses were performed for histological assessment, and parametric analyses were performed for bone morphological measures.

RESULTS: The groups that were administered sTNFRII and sTNFRII+IL-1Ra showed a detrimental effect on bone morphology, cartilage degeneration, and synovial inflammation. However, the IL-1Ra group reduced cartilage degeneration at 4 and 8 weeks and showed no detrimental effects on bone morphology. IL-1Ra was the only group in which the Mankin score of the fractured limb was not significantly different from the control limb (Figure 1). Additionally, IL-1Ra significantly reduced synovial inflammation at 8 weeks. IL-1Ra was the only group in which synovitis scores of fractured limbs were not significantly different than control limbs (Figure 2).

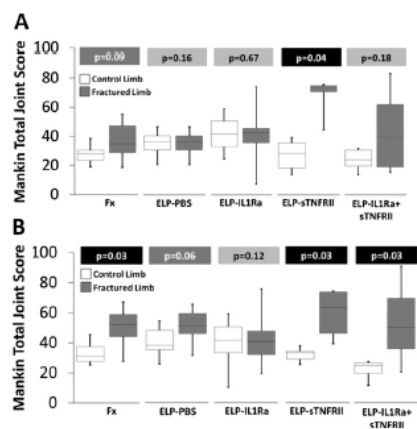


Figure 1. Mankin total scores at (A) 28 days and (B) 56 days post-fracture for all treatment groups. P values denotes significance between control (R) and fractured (L) limb Wilcoxon Matched Pairs test.

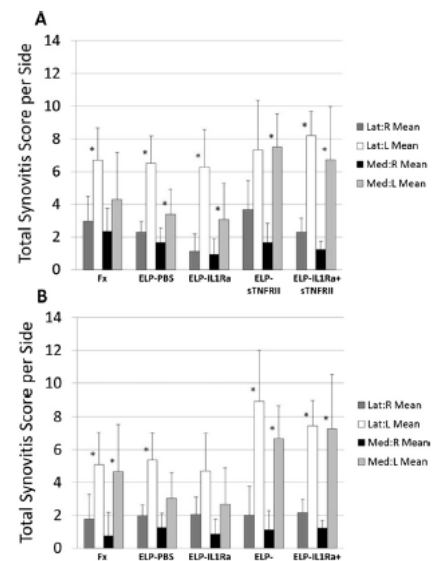


Figure 2. Synovitis scores of medial and lateral regions of knee joint capsule for non-fractured control (R) and fractured (L) limbs at (A) 28 days post-fracture and (B) 56 days post-fracture for all treatment groups. * denotes significance (p<0.05) between fractured and control limb using Wilcoxon Matched Pairs test.

CONCLUSIONS: The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue. However, the inhibition of TNF- α resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue. This study shows a novel reduction in post-trauma inflammation and demonstrates utility for an injectable drug depot for clinical intra-articular applications in the treatment of joint trauma.

Appendix II – Published Abstract of OARSI Meeting

S30

Abstracts / Osteoarthritis and Cartilage 21 (2013) S9–S62

Incidence of Lesion and Erosion Site Occurrence		
	Average # of Lesions	Average # of Erosion Sites
Control	0	0
MMT + saline	2.4 ± 0.7	2.8 ± 0.2
MMT + Amnion	0	1.2 ± 0.37

43 PROLONGED LOCAL DELIVERY OF IL-1RA PREVENTS POST-TRAUMATIC ARTHRITIS IN MICE

K.A. Kimmerling[†], B.D. Furman[†], D.S. Mangiapani[†], M.A. Moverman[†], S.M. Sinclair[†], J.L. Huebner[†], V.B. Kraus[†], L.A. Setton^{†,‡}, E. Guilak^{†,‡}, S.A. Olson[†], [†]Duke Univ. Med. Ctr., Durham, NC, USA; [‡]Duke Univ., Durham, NC, USA

Purpose: Post-traumatic arthritis (PTA) is defined as osteoarthritis (OA) resulting from joint trauma. Pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF- α) are reported to be upregulated following joint trauma and have been implicated in the pathogenesis of PTA. Presently, surgical restoration is the only treatment for articular fractures. We hypothesize that local sustained inhibition of IL-1, TNF- α , or IL-1 and TNF- α together prevents the development of post-traumatic arthritis following articular fracture. Our objectives were to locally inhibit early intra-articular inflammation following articular fracture in the mouse knee and assess the severity of OA changes in cartilage and joint tissues long-term.

Methods: All animal procedures were performed in accordance with an IACUC-approved protocol. Male C57BL/6 mice (n=77) were subjected to an articular fracture at 16 weeks of age using an established model. Five groups were established (n=12-16 per group). One group received no treatment after fracture. The remaining groups received intra-articular injections of PBS, IL-1 Receptor antagonist (IL-1Ra; anakinra; Kineret®), soluble TNF receptor II (sTNFRII; entanercept; Enbrel®) or both IL-1Ra and sTNFRII in combination. The treatments were encapsulated in elastin-like polypeptide (ELP) drug depots that have been associated with a 25-fold increase in half-life for intra-articular delivery.

Mice (n=6-8 per group) were sacrificed at 4 and 8 weeks. The left (fractured) and right (non-fractured) limbs were harvested and fixed. Micro-computed tomography (microCT) of both limbs was performed to assess bone morphology. Joints were processed for standard histology, and sections were assessed by 3 independent blinded graders for cartilage degeneration using a modified Mankin score and synovial inflammation using a modified synovitis score with semi-quantitative scales. Non-parametric statistical analyses were performed for histological assessment, and parametric analyses were performed for bone morphological measures.

Results: The groups that were administered sTNFRII and sTNFRII+IL-1Ra showed a detrimental effect on bone morphology, cartilage degeneration, and synovial inflammation. However, the IL-1Ra group reduced cartilage degeneration at 4 and 8 weeks and showed no detrimental effects on bone morphology. IL-1Ra was the only group in which the Mankin score of the fractured limb was not significantly different from the control limb (Figure 1). Additionally, IL-1Ra significantly reduced synovial inflammation at 8 weeks. IL-1Ra was the only group in which synovitis scores of fractured limbs were not significantly different than control limbs (Figure 2).

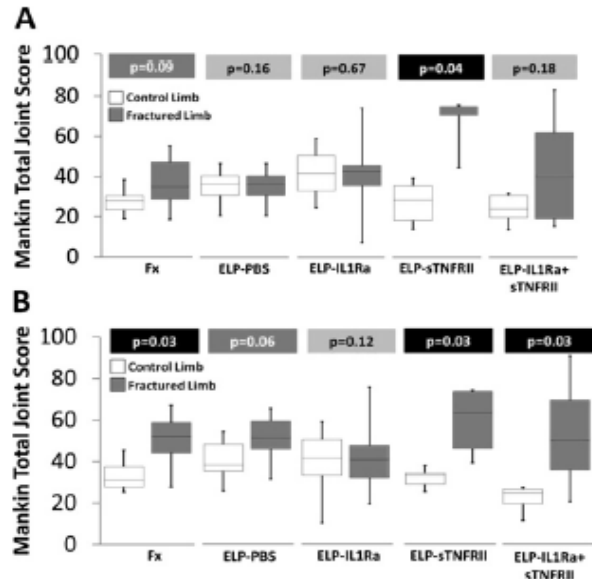


Figure 1. Mankin total scores at (A) 28 days and (B) 56 days post-fracture for all treatment groups. P values denotes significance between control (R) and fractured (L) limb Wilcoxon Matched Pairs test.

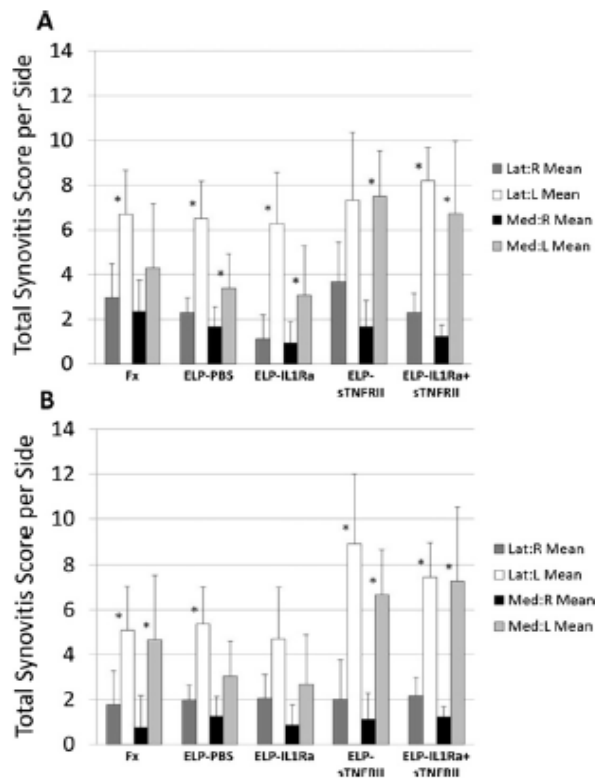


Figure 2. Synovitis scores of medial and lateral regions of knee joint capsule for non-fractured control (R) and fractured (L) limbs at (A) 28 days post-fracture and (B) 56 days post-fracture for all treatment groups. * denotes significance (p<0.05) between fractured and control limb using Wilcoxon Matched Pairs test.

Conclusions: The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue. However, the inhibition of TNF- α resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue. This study shows a novel reduction in post-trauma inflammation and demonstrates utility for an injectable drug depot for clinical intra-articular applications in the treatment of joint trauma.

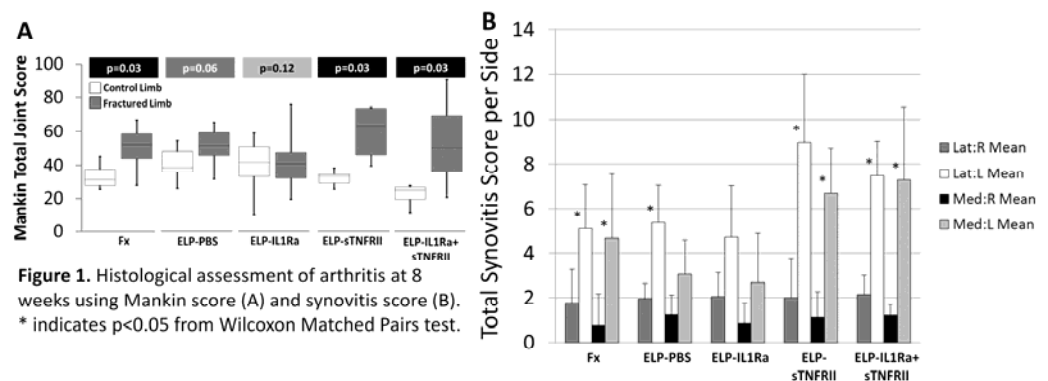
Sustained Intra-articular Delivery of IL-1Ra from a Thermally Responsive Polypeptide Depot Prevents Post-traumatic Arthritis in Mice

Kelly A. Kimmerling, MEng (n); Bridgette D. Furman, BS (n), Daniel S. Mangiapani, BS (n); Michael A. Moverman (n); S. Michael Sinclair, MS (n); Janet L. Huebner, MS (n); Virginia B. Kraus, MD/PhD (disclosures); Lori A. Setton, PhD (disclosures); Farshid Guilak, PhD (disclosures); Steven A. Olson, MD (disclosures) Duke University Medical Center & Duke University, Durham, NC USA

PURPOSE: Pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF- α) are reported to be upregulated following joint trauma and have been implicated in the pathogenesis of post-traumatic arthritis (PTA). Presently, surgical restoration is the only treatment for articular fractures. We hypothesize that local sustained inhibition of IL-1, TNF- α , or both IL-1 and TNF- α prevents the development of PTA following articular fracture.

METHODS: Animal procedures were performed in accordance with an IACUC-approved protocol. Male C57BL/6 mice (n=77) were subjected to an articular fracture at 16 weeks of age using an established model. Five groups were created (n=12-16). One group received no treatment after fracture (Fx). The remaining groups received intra-articular injections of PBS, IL-1 Receptor antagonist (IL-1Ra; anakinra; Kineret®), soluble TNF receptor II (sTNFR_{II}; etanercept; Enbrel®) or both IL-1Ra and sTNFR_{II}. The drugs were encapsulated in elastin-like polypeptide (ELP) drug depots that slowly disaggregate for prolonged IA delivery. Mice (n=6-8 per group) were sacrificed at 4 and 8 weeks. The left (Fx) and right (Non-Fx) limbs were harvested and fixed. Micro-computed tomography (MicroCT) of both limbs was performed to assess bone morphology. Histological sections of joint tissue were assessed for cartilage degeneration using a modified Mankin score and synovial inflammation using a modified synovitis score. Non-parametric statistical analyses were performed for histological assessment, and parametric analyses were performed for bone morphological measures.

RESULTS: Both groups that received sTNFR_{II} showed a detrimental effect in bone morphology, cartilage degeneration, and synovial inflammation. However, the IL-1Ra group reduced both cartilage degeneration (Fig 1A) and synovial inflammation (Fig 1B).



CONCLUSIONS: The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue. However, the inhibition of TNF- α resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue. This study shows a novel method of post-trauma inflammation relief that has the potential for use in intra-articular clinical settings.